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ORIGINAL ARTICLE

A New Species of the Genus *Thermophis* (Serpentes: Colubridae) from Shangri-La, Northern Yunnan, China, with a Proposal for an Eclectic Rule for Species Delimitation

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Abstract The hot-spring snakes, *Thermophis*, were previously known only from the Tibet Autonomous Region and Western Sichuan, China. During the past two years, three adult hot-spring snakes (2 females, 1 male) were sampled in Shangri-La, northern Yunnan, China, thus expanding their known distribution region towards the southeast. This site is the southeastern-most corner of the Tibetan Plateau and the southernmost tip of the Hengduan Mountains (Mts.). Phylogenetic analysis of mitochondrial (mt) and nuclear (n) DNA segments suggested that the three specimens belong to the genus of *Thermophis*. Morphologically, the new species is more similar to *T. zhaoermii*. However, it is distinguished from *T. zhaoermii* in the number of maxillary teeth (15), distance between the two eyes/head width, rostral width/height, mental width/height, in one character limited to female: head width/length, and in four characters restricted to male: occurrence of the reduction from 10 to 8 (8 to 6, 6 to 4) scales in each dorsal row on the tail. There are differences in morphology, genetics (mtDNA, nDNA), and geography between the putative new species and *T. zhaoermii,* the new species meets our proposed eclectic and feasible "four-differences" rule.

Keywords morphology, phylogenetics, "four-differences" rule, *Thermophis Shangrila* sp. nov.

1. Introduction

Despite more than one century of effort, taxonomists still have been trying to reach a consensus on the concept of species and methods of species delimitation (Mayden, 1997; de Queiroz, 1998; Fu and Zeng, 2008; Yang and Rannala, 2010; Chen *et al*., 2013b). To determine the validity of a species, relaxed criteria may result in confusion, whereas overly strict criteria may not facilitate consistent communications and actions in the practice of taxonomy and conservation biology. Here, we propose an eclectic and feasible rule—viz., the "four-differences" rule—to determine the validity of a species, also apply to identify a new species. First and foremost, the morphological difference(s) compared with the closest species should be perceptible. To further confirm that the morphological difference(s) possess taxonomic significance rather than representing intraspecific polymorphism, we also need evidence of mitochondrial DNA (matrilineal divergence) and nuclear DNA differences (patrilineal divergence). Finally, the presence of geographical or ecological difference represents the potential for natural reproductive isolation. In this paper, we illustrate the utility of this rule in relation to the taxonomy of hot-spring snakes.

Hot-spring snakes, *Thermophis*, a relict genus endemic to the Tibetan Plateau, achieve the world's highest altitude distribution among all snakes, commonly restricted to the proximity of geothermal sites (Zhao, 2006; Dorge *et al*.,

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2007; Huang *et al.*, 2009; He *et al.*, 2009; Hofmann, 2012). This genus was erected by Malnate (1953), and only included one species (*Thermophis baileyi* Wall, 1907) at that time, which was restricted to the Tibet Autonomous Region (TAR). The species was listed as "vulnerable" (IUCN, 2010).

Dorge *et al*. (2007) reported 13 new distribution records of *T. baileyi* in TAR, as well as predicting that if suitable habitats were available, *Thermophis* could also occur in hot springs in Sichuan and probably even in the northern parts of Yunnan. Guo *et al*. (2008) described a new species, *T. zhaoermii* in Cogsum, western Sichuan.

During investigations of biogeography and taxonomy of snakes in Western China in summer for the past two years (2011, 2012), we sampled three adult snakes (2 females, 1 male) in the vicinity of a hot spring in Shangri-La, northern Yunnan (Figure 1 A). Based on morphological, molecular and geographical analyses, these three specimens, while clearly belonging to the genus *Thermophis*, differ from the other two known species and should be considered a new species of this genus.

2. Materials and Methods

All sampling and procedures involving live snakes were in accordance with the Wild Animals Protection Law of the People's Republic of China and approved by the Animal Ethics Committee at Huangshan University. The 3 adult snakes (2 females, 1 male) were humanely euthanized using lethal injection at our laboratory. Fresh liver tissues were removed and immediately preserved in 95% ethanol. The holotype (Collection number: HS11192) and paratypes (Collection number: HS12120–21) were preserved and deposited in the Museum of Huangshan University (Voucher numbers: HUM20120001–3).

We examined 23 characters from the 3 specimens, 12 *T. zhaoermii* and 11 *T. baileyi*. Fourteen scale counts were taken, for the numbers of preoculars, postoculars, temporals, supralabials, infralabials, loreal, dorsals, ventrals, subcaudals, nasals, subcaudal scale position of the reduction from 10 to 8 (8 to 6, 6 to 4) scale rows in tail, and the number of maxillary teeth. Nine mensural characters were represented as: total length, tail length, distance between the two eyes, head width, head length, rostral height, rostral width, mental height, and mental width. Body and tail lengths were measured using a ruler to the closest 1 mm; other measurements were measured with an electronic caliper to 0.1 mm. Symmetric mensural head characters were measured only on the right, while asymmetric characters were recorded on both sides.

Total genomic DNA was extracted according to the phenol/chloroform extraction procedure (Sambrook *et al*., 1989). Three mtDNA and one nDNA sequences of the three specimens, four *T. zhaoermii* and five *T. baileyi* were obtained by polymerase chain reaction (PCR) and direct sequencing using the primers and methods described in Che *et al*. (2012) for COI (611 bp), Burbrink *et al*. (2000) for cytb (1095 bp), Arévalo *et al*. (1994) for ND4 (651 bp), and Lawson *et al*. (2005) for c-mos (567 bp). The four DNA segments are frequently used in studies of snake phylogeny, and have been shown to

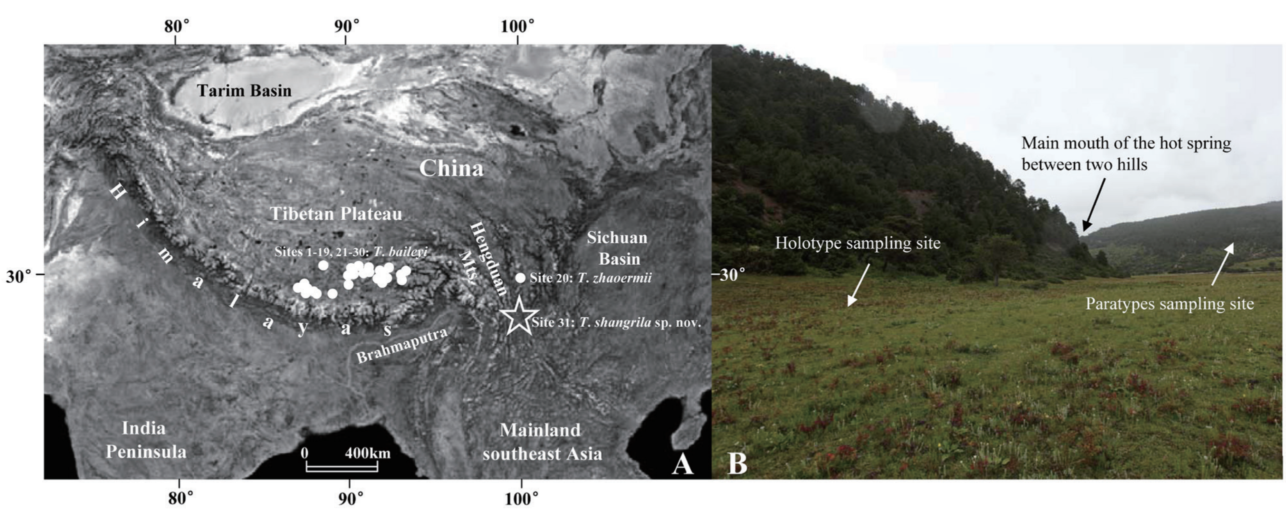

Figure 1 Sampling sites of *Thermophis* and habitat of *T. shangrila* sp. nov. A: The type locality (site 31, see Table 3) of *T. shangrila* sp. nov. is indicated by an asterisk, located at Shangrila, Northern Yunnan, China (N 27º28', E 99º29'; 3362 m a.s.l.). The other known sampling sites (1–30, see Table 3) of the genus *Thermophis* are shown by white circles. B: Habitat of type locality of *T. shangrila* sp. nov. Photo by S. HUANG.

resolve relationships well in every taxonomic category of snakes (Kraus and Brown, 1998; Burbrink *et al*., 2000; Kelly *et al*., 2003; Malhotra and Thorpe, 2004; Lawson *et al*., 2005; de Queiroz and Lawson, 2008; Dawson *et al*., 2008; Huang *et al*., 2009; Pyron *et al*., 2011, 2013; Chen *et al*., 2013a).

In order to find the molecular systematic position of the three snakes of this study, we retrieved cytb, ND4 and c-mos sequences from GenBank for 85 genera (Table 1) of Alethinophidia (primitive + advanced snakes). Among these, 24 species (from 12 genera and two different species each genus) only had cytb and/or ND4 and/or c-mos sequences, while 79 species (from 73 genera) had all three genes. In order to maximize our dataset of combined genes, three different sequences from two congeneric species were combined to form supraspecific terminals at the generic level (according to Bininda-Emonds *et al*., 1998; Kelly *et al*., 2003; Huang *et al*., 2009). We aligned the new cytb, ND4 and c-mos sequences of *Thermophis*, as well as other sequences retrieved from GenBank using Clustal X (Thompson *et al*., 1997). Bayesian Inference (BI) and Maximum likelihood (ML) methods were used to construct phylogenetic trees as described in Huang *et al*. (2009). In order to detect mtDNA differences and nDNA differences, uncorrected pair-wise distances and unrooted trees of all sequences of each of those four genes of *Thermophis* (from this study and Genbank) were calculated using PAUP* v4b10 (Swofford, 2002) and MEGA 4.0 (Tamura *et al*., 2007).

3. Results

Molecular genetic analyses

All three specimens shared one haplotype for each of the four genes. These sequences have been submitted to GenBank. Their accession numbers are KF038429 (CO1), KF038430 (cytb), KF038435 (ND4), KF514883 (c-mos). We also sequenced the four genes of four *T. baileyi* and five *T. zhaoermii*. For CO1 gene, four *T. baileyi* shared 3 haplotypes (accession numbers: KF038425–27), five *T. zhaoermii* shared 1 haplotype (accession number: KF038428). The sequences of Cytb, ND4 and c-mos all shared the same haplotypes with the sequences previously reported (Huang *et al*., 2009; He *et al*., 2009, 2010; Hofmann, 2012).

Bayesian Inference (BI) and Maximum likelihood (ML) methods were used to construct phylogenetic trees. The topologies of separate analyses of each mitochondrial gene (trees not shown), and combined genes (Figure 2) were nearly identical. The node supports of the trees based on single gene were generally low, especially for internal nodes. The topologies of combined genes of BI and ML bifurcating trees were almost identical. The basic relationships of the commonly recognized families and/or subfamilies are similar to Kelly *et al*. (2003), Pinou *et al*. (2004), Lawson *et al*. (2005) and Huang *et al*. (2009). The three snakes clustered in the genus *Thermophis* with clear internal structure and high supports.

For mitochondrial genes, the minimums of interspecific divergences are 4.1 times (CO1), 38 times (cytb) and 2.3 times (ND4) more than the maximums of intraspecific divergences (Figure 3 B, C, D). For nuclear gene c-mos, 3 haplotypes (from 14 sequences) represent 3 species respectively. No intraspecific divergence was found. The numerical values of pair-wise distances of 3 species seem well-distributed (Figure 3 A).

Taxonomy

***Thermophis shangrila* sp. nov.** (Figures 4, 5)

Shangri-La hot-spring snake, Xianggelila Wenquanshe (Bopomofo)

Holotype: HUM20120001, adult female, was captured on a grassland at the forest edge (see Figure 1 B) near a hot spring about 500 m in Shangri-La, Northern Yunnan, China, at 12:30 on 23 August, 2011, when it was fast moving towards the forest.

Paratypes: HUM20120002, adult male, was captured on the forest path of the opposite hill (see Figure 1 B) at 15:25, on 20 July, 2012, when it was basking on the path. HUM20120003, adult female, was captured on the same path at 18:29, on same day, when it was passing through the path.

Diagnosis: *Thermophis shangrila* sp. nov. is distinguished from all other species of *Thermophis* by number of maxillary teeth and external characters (Table 2). These three individuals all have 15 maxillary teeth, are distinguished from *T. baileyi* (21–24) and *T. zhaoermii* (16–17). *T. shangrila* sp. nov. is morphologically distinguished from similar species *T. zhaoermii* by several characters: a closer distance between the two eyes (distance between the two eyes/head width is 0.56 in females, 0.58 in male, *vs.* 0.59–0.61 in females, 0.64–0.73 in males), a wider rostral (rostral width/height is 1.70–2.40 in females, 2.10 in male, *vs.* 1.00–1.50 in females, 1.72–1.86 in males), and a wider mental (mental width/height is 1.85–2.05 in females, 1.45 in male *vs.* 1.45–1.77 in females, 1.15–1.30 in males). In females, a slightly longer head (head width/length is 0.62–0.65 *vs.* 0.68–0.71). In males, a more anterior occurrence of the reduction from 10 (8, 6) to 8 (6, 4) scales in each dorsal

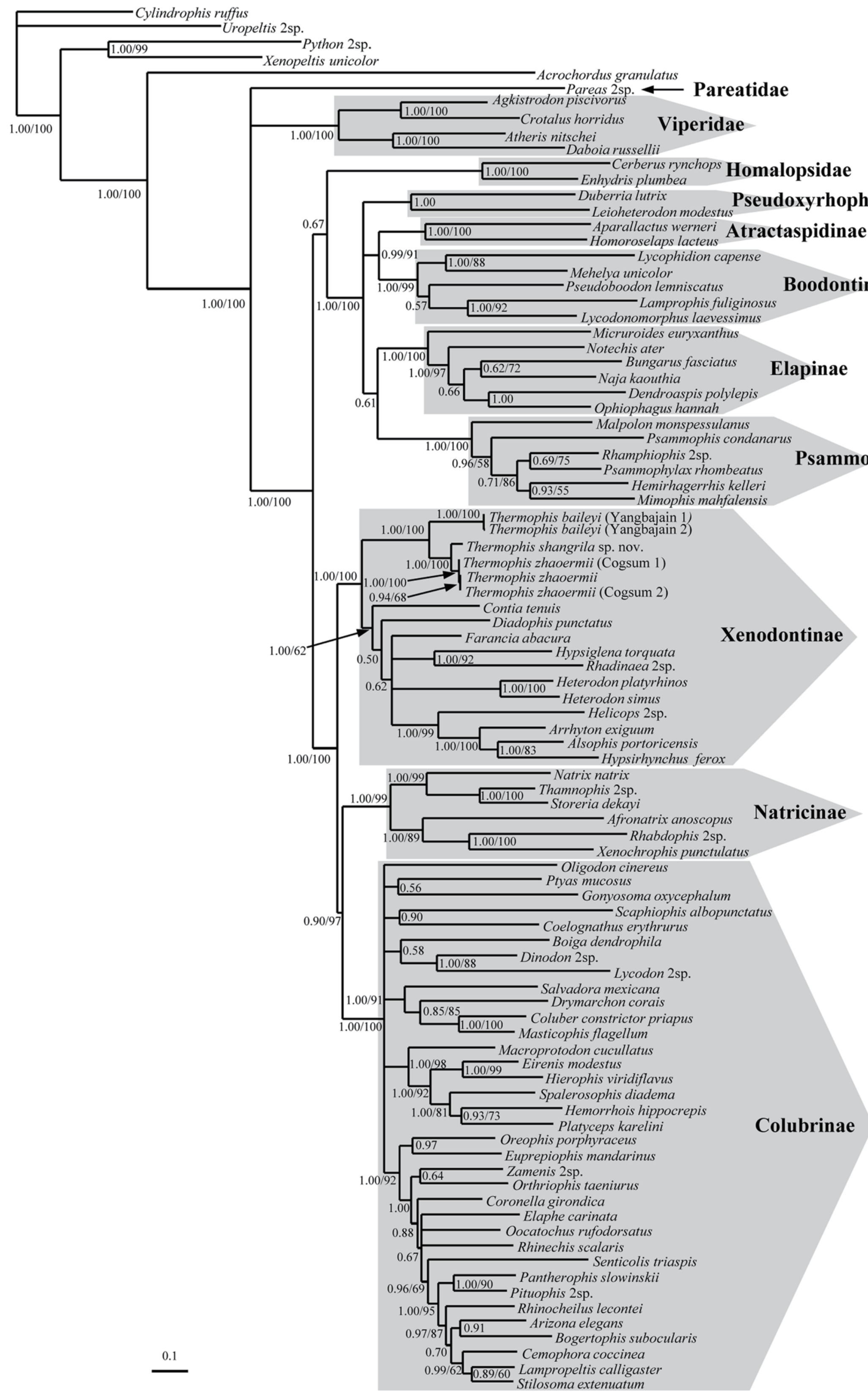

Figure 2 The 50% majority-rule consensus tree from Bayesian Inference analysis based on cytb, ND4 and c-mos combined sequences. The values on the corresponding branches indicate posterior probability support (BI)/bootstrap support (ML), under 50% from both analyses omitted. Likelihood settings from best-fit model (GTR + I + G) selected by AIC in jModeltest 0.1 (–ln L = 66699.4633). Rates = gamma, Shape = 0.4560, Pinvar = 0.3310. Supraspecific terminals are labeled with generic names + 2sp.

Table 1 Accession numbers of the sequences retrieved from GenBank and sequenced in this study. Systematic nomenclature follows the updated classification recommended by Lawson *et al*. (2005).

Family / Subfamily	Genus and species	Accession No. Cytb	ND4	c-mos
Acrochordidae	*Acrochordus granulatus*	AB177879	AB177879	EU366454
Colubridae				
Colubrinae	*Arizona elegans*	DQ902101	AF138750	DQ902058
	Bogertophis subcularis	DQ902103	DQ902281	DQ902060
	Boiga dendrophila	AF471089	U49303	AF471128
	Cemophora coccinea	AF471091	DQ902282	AF471132
	Coelognathus erythrurus	DQ902108	DQ902288	DQ902067
	Coluber constrictor priapus	AY486913	AY487040	AY486937
	Coronella girondica	AF471088	AY487066	AF471113
	Dinodon semicarinatus	AB008539	AB008539	—
	Dinodon rufozonatum	—	—	JF827695
	Drymarchon corais	AF471064	DQ902314	AF471137
	Eirenis modestus	AY486933	AY487072	AY486957
	Elaphe carinata	DQ902133	DQ902284	DQ902063
	Euprepiophis mandarinus	DQ902115	DQ902294	DQ902073
	Gonyosoma oxycephalum	AF471084	DQ902309	AF471105
	Hemorrhois hippocrepis	AY486916	AY487045	AY486940
	Hierophis viridiflavus	AY486925	AY487057	AY486949
	Lampropeltis calligaster	DQ902129	DQ902311	DQ902091
	Lycodon zawi	AF471040	—	KC010336
	Lycodon capucinus	—	U49317	—
	Macroprotodon cucullatus	AF471087	AY487064	AY187987
	Masticophis flagellum	AY486927	AY487060	AY486952
	Oligodon cinereus	AF471033	U49316	AF471101
	Oocatochus rufodorsatus	DQ902123	DQ902301	DQ902081
	Oreophis porphyraceus	DQ902118	DQ902298	DQ902076
	Orthriophis taeniurus	EF076709	EF076708	EF076705
	Pantherophis slowinskii	DQ523162	DQ523162	FJ627792
	Pituophis melanoleucus	DQ902130	DQ902312	—
	Pituophis ruthveni	—	—	DQ902092
	Platyceps karelini	AY486918	AY487047	AY486942
	Ptyas mucosus	AF471054	AY487063	GQ225670
	Rhinechis scalaris	AY486932	AY487068	AY486956
	Rhinocheilus lecontei	AF337109	AY138774	FJ627788
	Salvadora mexicana	AY486934	AY487075	AY486958
	Senticolis triaspis	DQ902127	AF138775	DQ902086
	Spalerosophis diadema	AF471049	AY487059	AY486950
	Stilosoma extenuatum	DQ902131	AF138776	DQ902093
	Zamenis longissimus	DQ902138	—	DQ902072
	Zamenis lineatus	—	DQ902319	—
Natricinae				
	Afronatrix anoscopus	AF420073	AF420076	AF471123
	Natrix natrix	AF471059	AY487800	AF544697
	Rhabdophis subminiatus	—	U49325	AF544713
	Rhabdophis tigrinus	AF471051	—	—
	Storeria dekayi	AF471050	EF417365	AF471154
	Thamnophis sirtalis infernalis	AF420193	AF420196	—
	Thamnophis sirtalis sirtalis	—	—	DQ902094
	Xenochrophis punctulatus	AF71079	AY87074	AF471106
Xenodontinae (Dipsadinae)				
	Diadophis punctatus	AF471094	AF258899	AF471122
	Hypsiglena torquata	AF471038	EF078548	AF471159
	Rhadinaea flavilata	AF471078	—	AF471152
	Rhadinaea fulvivittis	—	EF078587	—
Xenodontinae (Xenodontinae)				
	Arrhyton exiguum	AF471071	FJ416799	AF471117
	Alsophis portoricensis	AF471085	U49308	AF471126
	Contia tenuis	AF471095	AF258879	AF471134
	Farancia abacura	U69832	DQ902307	AF471141
	Helicops angulatus	AF471037	—	AF471160

(Continued Table 1)

Family Subfamily	Genus and species	Accession No.		
		Cytb	ND4	c-mos
	Helicops pictiventris	—	U49310	—
	Heterodon platyrhinos	GU112412	AF402659	JQ598986
	Heterodon simus	AF217840	DQ902310	AF471142
	Hypsirhynchus ferox	GQ895875	FJ416816	GQ895818
Colubridae *insertae sedis*				
	Thermophis zhaoermii (Cogsum 1)	EU864145	EU864150	KF514882
	Thermophis zhaoermii (Cogsum 2)	EU864146	EU864150	KF514882
	Thermophis zhaoermii	GQ166168	GQ166168	KF514882
	Thermophis baileyi (Yangbajain 1)	EU864147	EU864149	KF514884
	Thermophis baileyi (Yangbajain 2)	EU864148	EU864149	KF514884
	Thermophis shangrila sp. nov.	KF038430	KF038435	KF514883
Elapidae				
Atracaspidinae	*Aparallactus werneri*	AF71035	U49315	AF471116
Boodontinae				
	Lamprophis fuliginosus	AF471060	DQ486315	AF544686
	Lycodonomorphus laevessimus	DQ486338	DQ486314	DQ486162
	Lycophidion capense	DQ486344	DQ486320	AY611984
	Pseudoboodon lemniscatus	DQ486350	DQ486325	DQ486174
	Scaphiophis albopunctatus	DQ486345	DQ486321	DQ486169
Elapinae				
	Bungarus fasciatus	NC011393	NC011393	AY058924
	Dendroaspis polylepis	AF217832	AY058974	FJ387197
	Homoroselaps lacteus	AF217833	AY058976	AY058931
	Mehelya unicolor	AF471077	DQ486324	AF471099
	Micruroides euryxanthus	AF217823	EF137408	EF137423
	Naja kaouthia	DQ343648	DQ343648	AY058938
	Notechis ater	AF217836	AY058981	AY058937
	Ophiophagus hannah	EU921899	EU921899	AY058940
Psammophiinae				
	Hemirhagerrhis kelleri	DQ486335	DQ486311	DQ486159
	Malpolon monspessulanus	AY058965	AY058989	AY187990
	Mimophis mahfalensis	AY188032	DQ486202	AF544688
	Rhamphiophis acutusacutus	DQ486425	DQ486262	—
	Rhamphiophis oxyrhynchus	—	—	AF544710
	Psammophis condanarus	AF471075	AY058987	AF471104
	*Psammophylax rhombeatu*s	DQ486361	DQ486200	FJ404230
Pseudoxyrhophiinae				
	Duberria lutrix	AF471061	DQ486240	AF471138
	Leioheterodon modestus	AY058967	AY058978	AY058933
Pareatidae				
	Pareas macularius	AF471082	—	AF471150
	Pareas nuchalis	—	U49311	—
Viperidae				
Crotalinae				
	Agkistrodon piscivorus	DQ523161	DQ523161	AF471096
	Crotalus horridus	NC014400	NC014400	JN620897
Viperinae				
	Atheris nitschei	AF471070	AY223618	AF471125
	Daboia russellii	EU913478	EU913478	AF471156
Homalopsidae				
	Cerberus rynchops	AF471092	U49327	EF395926
	Enhydris plumbea	DQ343650	DQ343650	EF395934
Outgroups:				
Cylindrophiidae	*Cylindrophis ruffu*s	AB179619	AB179619	AF471133
Pythonidae	*Python regius*	AB177878	AB177878	—
	Python molurus	—	—	AY099968
Uropeltidae	*Uropeltis phillipsi*	AF471034	—	AF471100
	Uropeltis sp.	—	DQ904396	—
Xenopeltidae	*Xenopeltis unicolor*	AB179620	AB179620	DQ465561

Table 2 The comparison of distinguishing characters of the three species of *Thermophis*.

Characters	Female (mm)			Male (mm)		
	T. shangrila sp. nov. (*n* = 2)	*T. zhaoermii* (*n* = 10)	*T. baileyi* (*n* = 5)	*T. shangrila* sp. nov. (*n* = 1)	*T. zhaoermii* (*n* = 2)	*T. baileyi* (*n* = 6)
head width/ length	0.62–0.65	0.68–0.71	0.49–0.68	0.59	0.56–0.62	0.47–0.49
distance between the two eyes/head width	0.56	0.59–0.61	0.65–0.77	0.58	0.64–0.73	0.70–0.74
rostral width/height	1.70–2.40	1.00–1.50	1.05–1.44	2.10	1.72–1.86	1.26–1.67
mental width/height	1.85–2.05	1.45–1.77	1.39–1.44	1.45	1.15–1.30	1.37–1.40
subcaudal scale position of the reduction from 10 to 8 scale rows in tail	25–27	21–30	21–29	30	34.5–35	32–37.5
subcaudal scale position of the reduction from 8 to 6 scale rows in tail	46–42	43–54	51–59	49	52–61.5	55.5–67.5
subcaudal scale position of the reduction from 6 to 4 scale rows in tail	69–63	64–74.5	76–81.5	75	76.5–77	85.5–92
the number of maxillary teeth	15	16–17	21–24	15	16–17	21–24

n: the number of specimens

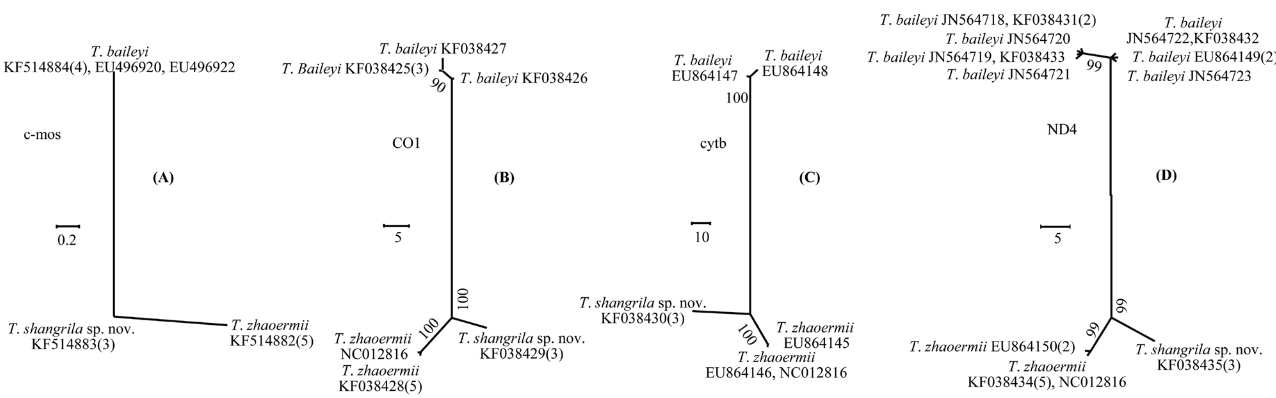

Figure 3 Unrooted trees and genetic distances of *Thermophis* were calculated based on the partial c-mos, CO1, cytb, and ND4 gene sequences derived from MP analyses. Numbers above or below branches are MP bootstraps values, only showing those higher than 50%. The number in parentheses indicate that the number of specimens that share this haplotype. (A) The *p*-distances of partial c-mos gene of 3 haplotypes of 14 sequences (each haplotype represents a species) of *Thermophis* ranged between 0.2%–0.5% among species. (B) The *p*-distances of partial CO1 gene of 6 haplotypes of 15 sequences of *Thermophis* ranged between 2.9%–11.2% among species, the maximum within species is 0.7%. (C) The *p*-distances of partial cytb gene of 5 haplotypes of 17 sequences of *Thermophis* ranged between 3.8%–12.6% among species, the maximum within species is 0.1%. (D) The *p*-distances of partial ND4 gene of 10 haplotypes of 23 sequences of *Thermophis* ranged between 2.5%–11.2% among species, the maximum within species is 1.1%. Sequences from this study; Huang *et al*., 2009; He *et al*., 2009; He *et al*., 2010; and Hofmann, 2012.

row on the tail (30 *vs*. 34.5–35, 49 *vs*. 52–61.5, 75 *vs*. 76.5–77).

Description of holotype and variation: When differing from the holotype, features of the paratypes follow in parentheses (in the order HUM20120002, HUM20120003). Holotype an adult female (adult male, adult female) with body weight 121 g (83 g, 105 g), snout-vent length 743 mm (589 mm, 659 mm), tail length 208 mm (206 mm, 186 mm). Scutellation: 2 preoculars; 2 postoculars; 2 + 3 temporals; 8 supralabials, the 4th and 5th bordering the eye; 10 infralabials; 1 loreal; 19–19–17 forebody-midbody-hindbody transverse dorsal scale rows, dosal scales well keeled, outer row smooth or faintly keeled posteriorly; 223 ventrals (212, 222); 88 pairs subcaudals (95, 92); anal plate divided. Body and headshape: body relatively slender; venter round; head narrow, elongate, not strongly distinct from the neck; eye large, pupils round; Coloration: the dorsal ground color is light-brown marked with dark chocolate spots and stripes. A vertebral stripe of dark gray-brown spots; posteriorly on the body the spots become obscure and the stripe more prominent. The scales of the first three rows are darker centrally and form a broad, smoky lateral stripe. Venter olive green; Central line on the ventral surface is

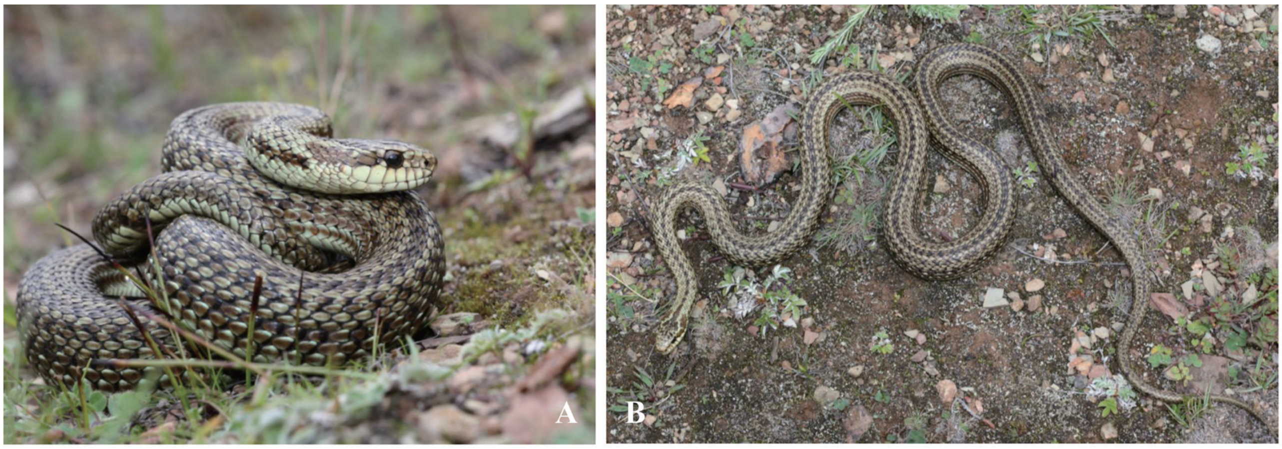

Figure 4 Body of Holotype (HUM20120001, adult female). A: Dorsolateral; B: Back. Photos by L. F. PENG.

Figure 5 Head of holotype (HUM20120001, adult female). A: Ventral; B: Dorsal; C: rostral and Right; D: Left. Photos by L. F. PENG.

prominent. Paler anteriorly and grayer posteriorly, the ventral plates narrowly edged with yellow laterally and along their free edges; the yellow lateral edge forms, with the yellow edge of the outer scale row, a narrow, irregular, ventro-lateral line. Each ventral with a pair of irregularly semicircular, black spots, which are more prominent on the posterior part of the belly (these are connected across the belly but the connecting areas of black are hidden by the overlapping edge of the preceding plate). Head brown, mottled with black-gray, lighter laterally and toward the snout. Supralabials creamy yellow, their sutures clouded dusky gray. Chin and throat yellow, the mental and anterior labials clouded gray-brown, the posterior labials narrowly edged with gray-green. Caudal pattern similar to that of the body, but much reduced.

Ecology: The species is a diurnal terrestrial snake.

Etymology: The specific name refers to the type locality, Shangri-La County, Yunnan, China.

4. Discussion

The coordinates and altitudes of the 31 currently known distribution sites of hot-spring snakes (according to the literature and our expeditions) are listed in Table 3. The discovery reported here expands the distribution region of hot-spring snakes in a southeasterly direction. The new site is the southeastern-most corner of the Tibetan Plateau and the southern-most tip of the Hengduan Mts. (Figure 1 A). The name "Hengduan" means "to transect" and "cut downward" in Chinese. Topographically, in this area, parallel mountain ranges are separated by deep, narrowly incised river valleys. With strongly geographic

Table 3 Geographic coordinates and Temperature of thermal springs of the known sampling sites of the genus *Thermophis*.

	Sites	Latitude	Longitude	Altitude (m)	Temperature (°C)	Source
1	Shanza Derha	30°54'	88°42'	4680	51.5	Dorge *et al.* (2007);
2	Nam Tso	30°36'	91°15'	4333	67	Sylvia (2012)
3	Ningtrong	30°24'	90°58'	4215	87	Sylvia (2012)
4	Damshong	30°23'	91°25'	4260	unknown	Dorge *et al.* (2007)
5	Nyanbo	30°12'	93°10'	3928	43	Sylvia (2012)
6	Dedrom	30°09'	92°10'	4418	67	Sylvia (2012)
7	Terdrom	30°09'	92°09'	4412	42.8	Dorge *et al.* (2007);
8	G109	30°09'	90°39'	4887	42	Sylvia (2012)
9	Yukchong	30°09'	90°39'	4891	85	Dorge *et al.* (2007);
10	Lower Terdrom	30°06'	92°10'	4401	42.8	Dorge *et al.* (2007);
11	Yangbajain	30°04'	90°29'	4100	40–90.5	Huang *et al.* (2009)
12	Yangbajain	29°51'	90°21'	4395	67	Sylvia (2012)
13	Yangjian	29°58'	90°21'	4410	77.5	Dorge *et al.* (2007);
14	Sumdo	29°54'	92°3'	4167	67	Sylvia (2012)
15	Gonbo Gyamsdha	29°53'	92°22'	4470	67	Dorge *et al.* (2007);
16	Gonbo Gyamsdha	29°53'	93°15'	3430	43	Huang *et al.* (2009 expedition)
17	Gonbo Gyamsdha	29°44'	92°21'	3650	67	Huang *et al.* (2012 expedition)
18	Ninang	29°44'	93°21'	3652	unknown	Sylvia (2012)
19	Rutok	29°42'	92°14'	4382	81.5	Sylvia (2012)
20	Cogsum*	29°41'	100°23'	3700	53	Liu and Zhao (2004); Huang *et al.* (2009)
21	Welka	29°37'	92°32'	3600	>45	Dorge *et al.* (2007)
22	Ngamring	29°23'	87°22'	4155	52.5	Sylvia (2012)
23	Xumai	29°28'	90°14'	4278	43	Miao He (2009 personal communication)
24	Tori	29°09'	87°12'	4120	44.5	Dorge *et al.*(2007)
25	Xiqin	29°04'	87°44'	4013	55	Huang *et al.* (2008 expedition)
26	Jiangtse Gyingka	28°42'	89°12'	4200	57	Dorge *et al.* (2007)
27	Manga	28°55'	87°34'	4597	47	Sylvia (2012)
28	Qiuku	28°54'	87°34'	4614	47	Huang *et al.* (2008 expedition)
29	Sagya Kou	28°54'	88°00'	4320	82	Dorge *et al.* (2007)
30	Sakya Kau	28°50'	88°10'	4602	82	Hofmann (2012)
31	Shangri-La#	27°28'	99°29'	3362	56	This study

*: in Sichuan province. #: in Yunnan Province. Others: all in Tibet Autonomous Region.
According to Dorge *et al*., 2007, the coordinates are given only with a precision of 10 minutes to avoid private or commercial sampling. The early papers aren't listed because of the lack of specific sites and coordinates available. The temperature data cited from Guo *et al*. (1994) and Tong *et al*. (2000).

heterogeneity, Hengduan Mts. had naturally resulted in vicariance restricting gene flow (Huang *et al*., 2009). Such geographical difference could represent the potential for natural reproductive isolation. There are differences in morphology, mtDNA, nDNA, and geography between the new species and *T. zhaoermii*. This new species meets our proposed eclectic and feasible "four-differences" rule.

In the Late Pliocene, the Qinghai-Xizang region (Tibetan Plateau), with an elevation of only about 1000 m a.s.l. (Li *et al*., 1979) was characterized by landscapes of subtropical montane forest and forest-steppe (Zheng and Li, 1990). At that time, the environment of this region should have facilitated the existence of many species of snakes. This region underwent intensive uplifting, beginning at the end of Pliocene and Early Pleistocene, and the environment changed gradually from warm-humid to cold-arid (Zheng and Li, 1990), reducing its ability to support populations of ectotherms. In the central region of the Tibetan Plateau (CRTP, between Transhimalaya and Tanggula Mts., west of Sejila Mts.), *Thermophis baileyi* is the only snake survivor currently known, and is exclusively restricted to habitats with hot springs (Dorge *et al*., 2007; Huang *et al*., 2009), thus making it an ideal model for the study of adaptive evolution in Serpentes.

The temperatures recorded at the mouths of hot springs at the 31 known sampling sites of *Thermophis* exceed 40°C (see Table 3, the temperature data cited from Guo *et al*., 1994 and Tong *et al*., 2000). There are 229 hot springs with the temperature hotter than 40°C in the CRTP (counted from Tong *et al*., 2000), therefore begging the question why only a few hot springs are utilized by *Thermophis*. Why did snakes sympatric with *Thermophis* in earlier geologic periods fail to survive in these regions? These observations raise many issues of considerable interest.

Uplifting of the Tibetan Plateau and incision of river valleys on its eastern margin must have occured simultaneously. Differing considerably from the CRTP, the deep incised valleys of the eastern margin of Tibetan Plateau have strong habitat heterogeneity, and offered refuges for many species (e.g., the famous Hengduan Mts.). This region harbors about 30 species of snakes, including *T. zhaoermii* and *T. shangrila* sp. nov. There are more than 400 hot springs hotter than 40°C in Hengduan Mts. (based on Guo *et al*., 1994), yet *Thermophis* have been found at only two of them.

Several fieldworks had been conducted from 2011 to 2014 in northern Yunnan and southern Sichuan. We found that the quality of habitats around the hot springs has declined because of the increasingly serious exploitation of geothermal energy. The habitat faces an extremely high risk of extinction in the wild. During these fieldworks, only one extent of occurrence of hot-spring snakes had been found, where we had got three samples (in 2011 and 2012). The number of individuals considered to be facing a continuing decline. According to IUCN Red List Categories and Criteria (Version 3.1, Second edition, 2001), the new species should be listed as "Critically Endangered" (A2cd+3cd+4cd; B1ab (i, iii, v)).

Taxonomy is an important foundation of biology. However, there are few taxonomists in China. Therefore, the National Natural Science Foundation of China has a focus on taxonomy in order to encourage its development (Chen *et al*., 2010). This is also a global phenomenon (Dr. Anita Malhotra, personal communication) where there are millions of species still undescribed and there are far too few taxonomists to do the job, especially in biodiversity-rich but economically poorer countries (http://www.cbd.int/gti/problem.shtml, web of Convention on Biological Diversity). Once it has undergone comprehensive discussion and improvement, we hope that it will help to promote the development of taxonomy and accelerate the description of new species.

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BOOK REVIEW

Salamanders of the Old World, Max Sparreboom. KNNV Publishing (2014). ISBN: 9789050114851

Currently nine families of tailed amphibians, including 682 species, are recognised in the world. Five of these families, including some 166 species, occur in the Old World (Europe, Asia, and Northern Africa). The family of hynobiid salamanders (hynobiidae) is endemic to this region, whereas the other families also are represented in the New World (America). *Salamanders of the Old World* is a comprehensive monograph authored by Max Sparreboom. With this book, which aims to be an up-date of Robert Thorn's book of 1969, the author presents updated information on salamander species diversity in this region. The book is bound to become a classic in its field: it contains descriptions of all five families and 166 salamander species, a taxonomical diagnosis, distribution maps, and a wealth of information on habitat preferences, behaviour, threats, conservation, etc. Every species chapter is followed by a comment by the author and references to technical literature. For many species the biological information comes from the author himself, who made observations of behaviour in aquariums and in the field. This knowledge is supplemented with information from the scientific literature, which is synthesized in a systematic way. What will especially appeal to the reader is the great number of beautiful pictures in the text, showing details of morphological characters, habitat and behaviour in the different life history stages. These features add to the value of the book, both in scientific and artistic aspects.

There is an increasing interest in the phenomenon of amphibian population declines in many parts of the world. Indeed, the decline of salamanders may be more serious than that of other amphibians. This book therefore comes at the right time. As a text book and reference, it is not only invaluable for the herpetologist, naturalist and conservationist, but it is also very useful for amateurs, who wish to identify and enjoy these fascinating creatures. The important effect of this book is that it sends a convincing message that salamanders deserve further study and conservation.

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